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14. ABSTRACT

This training grant is a Postdoctoral Fellowship Award in breast cancer research, comprising training in breast cancer research and an independent research project. During the previous year, the PI gained valuable experience in designing and conducting molecular epidemiologic studies and participated in various research projects as a leader or a collaborator. She has also acquired knowledge on breast cancer biology through various venues, incorporating new understanding of breast cancer etiology in the analyses of epidemiologic data. These experiences have helped in preparation for an independent research career. The research component of this grant involves conducting a case-cohort analysis to investigate the major determinants of urinary prostaglandin E_2-metabolite (PGE-M) and how the urinary level of PGE-M interacts with estrogen biosynthesis in relation to breast cancer risk in postmenopausal women. Urinary levels of PGE-M and estrogens (Es)/estrogen metabolites (EMs) are being measured using novel liquid chromatography/tandem mass spectrometry techniques. As of January 2012, quality control experiments have been completed for both PGE-M and Es/EMs, yielding promising data with satisfactory sensitivity and reliability. As for PGE-M, laboratory analysis of all 607 study samples has recently been completed, and preliminary data analysis found significant positive associations with levels of PGE-M for obesity, current smoking, and history of diabetes and cardiovascular diseases, after adjustment for other factors. As described in the Request for an Extension without Funds (EWOF), however, estrogen analysis is still in progress, with an expected completion date of the end of April 2012. Upon completion of the laboratory analysis, the PI will conduct a full analysis to investigate the interrelationships between PGE-M, estrogens and postmenopausal breast cancer risk, in accordance with the approved Statement of Work for this grant.

15. SUBJECT TERMS

Breast Cancer; Inflammation; Prostaglandin; Estrogen

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Introduction

This training grant is a Postdoctoral Fellowship Award in breast cancer research. The project involves focused mentorship and training in breast cancer research, with a particular focus on molecular epidemiology of breast cancer. During the previous year, the PI performed several research activities that further developed necessary skills and knowledge to independently carry out innovative multi-disciplinary breast cancer research. These include 1) quantitative assessment of bioassays in epidemiologic research setting; 2) incorporation of breast cancer biology in epidemiologic data analyses; and 3) development of new research projects.

The research segment of this grant is leading a case-cohort study within the Sister Study, a large prospective cohort study of women with family history of breast cancer, to investigate the major determinants of urinary prostaglandin E_2-metabolite (PGE-M) and how the urinary level of PGE-M interacts with estrogen biosynthesis in relation to breast cancer risk in postmenopausal women. At the beginning of the project, the PI experienced administrative delay in obligating and transferring funds to a contract laboratory. Moreover, the PI and her mentor decided to switch to a novel method for estrogen quantification because of scientific merit and sample efficiency (justified in the 2010 Annual Summary). Despite those unexpected events, the proposed project has made significant progresses during the last year, and the PI has learned valuable lessons on how to achieve the best possible results in unplanned circumstances.

Body

The objective of the proposed study was to determine how urinary levels of PGE-M interact with estrogen biosynthesis and influences breast cancer risk in postmenopausal women. A case-cohort analysis nested in a large national cohort of women was composed of 607 postmenopausal women who were aged 50 years or older and did not report current use of hormones, including 310 incident breast cancer cases (246 estrogen receptor [ER] positive; 40 ER negative, and 24 unknown ER status). Levels of PGE-M and estrogen metabolites were or are being quantified using novel liquid chromatography/tandem mass spectrometry techniques; therefore, additional quality control experiments were conducted using internal funds to ensure sufficient levels of assay performance to address the proposed study aims.

As of January 2012, all planned PGE-M analysis was completed. As described in the Request for an Extension without Funds (EWOF), however, estrogen analysis is still in progress, and is expected to be complete by the end of April 2012. Hence, this present Annual Report summarizes results from the quality control experiments of PGE-M and estrogen metabolites, and preliminary results of PGE-M analysis. Briefly, within- and between-batch % coefficients of variations (%CV) for PGE-M were 8% and 19%, which are within the acceptable range (Table 1).

A short-term reliability of PGE-M measurements was also estimated using 100 samples from 12 individuals who provided urine samples at three time points across 9 months. After controlling for batch effect, 64% of the total variance in PGE-M measurements was attributed to between-subject variability (Table 2). However, the short-term reliability was reduced to approximately 42% for creatinine-adjusted PGE-M measurements, regardless of adjustment for batch effect.

Out of 607 study samples for the case-cohort analysis, PGE-M levels were obtained in 604 women with geometric mean of 5.4 ng/mg Cr. (95% CI: 5.1-5.7). Signal for PGE-M was not detected in 3 subjects even after reanalysis. One of the proposed study aims was to identify factors affecting urinary levels of PGE-M in postmenopausal women. In our preliminary analysis, obesity, current smoking and history of diabetes and cardiovascular diseases were independently associated with higher levels of PGE-M (all p-values < 0.05, except for history of cardiovascular diseases with $p=0.08$) [Table 3]. On the other hand, women with rheumatoid arthritis had lower levels of PGE-M compared to those who didn't report having the condition (adjusted mean PGE-M: 5.5 vs. 4.4; $p=0.06$), which is postulated to be related to long-term use of nonsteroidal anti-inflammatory drugs. Age was not associated with PGE-M levels.

As for estrogen analyses, the results of the recently-completed quality control experiments are promising, yielding excellent %CVs (2.7% for parent estrogens; 5.9% of C2-hydroxylated estrogen metabolites [EMs], 7.1% for C4-hydroxylated EMs, 15.4% for C4-methlated EMs; and 8.4% for C16-hydroxiylated EMs)[Table 4b]. Statistical analyses to address the study aims as well as laboratory analysis of estrogens in the case-cohort sample are currently ongoing. It is expected that the present study will achieve the objectives of the study, contributing to understanding the role of inflammation in estrogen biosynthesis and breast cancer risk in postmenopausal women.

Key Research Accomplishments

Progress in proposed research project

- Prior to the analysis of study samples for PGE-M, additional quality control experiments were conducted using 100 samples from 12 women who provided urine samples at three different time points over 9 months. Samples were arranged across 5 batches to assess not only the reliability of the assay but also a short-term reproducibility of PGE-M measurement within individuals over time.

- Laboratory analyses of 607 study samples for PGE-M and creatinine were completed in November 2011. However, selected batches of samples showed relatively poor reproducibility. We believe this was due to equipment maintenance issues that were subsequently resolved. Reanalysis of affected batches (along with samples from earlier batches for quality control purposes) has been also recently completed with satisfactory results.
- For estrogen analysis, we have collaborated with the only NCI-licensed laboratory to ensure adequate levels of sensitivity and reliability of the assays. A series of quality control experiments followed by regular conference calls between the NIEHS investigators and chemists at the contract laboratory were conducted to detect source of technical errors. The second QC experiment results became available the first week of January 2012, showing sufficiently high sensitivity and reliability to move forward to analyze our study samples.

Training

- Had regular meetings with mentors (Drs. Dale Sandler and Jack Taylor) to discuss the research project, ongoing training, other related projects and strategies for progress of the project and future work

- Attended seminars at the NIEHS and national conferences such as 1) A Joint Meeting between AACR-ACS on Chemistry in Cancer Research: The Biological Chemistry of Inflammation as a Cause of Cancer in San Diego, CA between January 30 and February 2, 2011; and 2) Department of Defense Era of Hope Meeting in Orlando, Fl on August 2-5, 2011.

- Co-mentored a postbaccalalaureate student, Jean Strelitz at NIEHS preparing a poster on the stability of mitochondrial DNA copy number measurements. The poster was presented at Postbaccalaureate Research Festival at D.C. in May, 2011.
- Served as a reviewer for several peer-reviewed Journals including *American Journal of Epidemiology*, *American Journal of Clinical Nutrition*, *British Journal of Cancer*, *Sleep*, and *Clinica Chimica Acta*.

Other breast cancer research projects

- Reported that telomere length in peripheral blood was not associated with breast cancer risk in the prospective Sister Study cohort.

- Published a research paper that examined the reliability of telomere length measurements using sequential samples collected over a 9-month period. This manuscript reported good short-term reliability of telomere length measurements using blood from a single blood draw. However, the existence of technical variability, particularly batch effect, reinforces the need for technical replicates and balancing of case and control samples across plates.
- Readied two manuscripts for submission. The first manuscript about genetic variants in DNA and histone methylation and telomere length has been submitted for publication. A manuscript on microsatellite instability in relation to survival of African American and white patients with colorectal cancer, which was a collaborative project with Drs. Keku and Sandler at UNC, has also been submitted.
- Drafted a new research proposal on breast cancer and autoimmune diseases in women with endometriosis in the Sister Study
- Completed preliminary analysis of Sister Study data to examine the potential for nonsteroidal anti-inflammatory drugs to modify relationships between timing of reproductive factors and breast cancer.
- Began interviewing for tenure-track positions at the NIH, Comprehensive Cancer Centers, Schools of Public Health, and medical schools.

Reportable Outcomes

Manuscripts

Kim S, Parks CG, Xu Z, Carswell G, DeRoo LA, Sandler DP, Taylor JA. Association between genetic variants in DNA and histone methylation and telomere length. *Submitted*

Kim S, Sandler RS, Sander DP, Martin C, Galanko J, Keku TO Microsatellite Instability and colorectal cancer survival in African Americans and whites. *Submitted*

Kim S, Sandler DP, Carswell G, Cawthon RM, Weinberg CR, Taylor JA (2011). Reproducibility and short-term intra-individual variability of telomere length measurement using a monochrome multiplexing quantitative PCR. PLoS One 6(9):e25774

Kim S, Sandler DP, Carswell G, DeRoo LA, Parks CG, Cawthon RM, Weinberg CR, Taylor JA (2011). Telomere Length in Peripheral Blood and Breast Cancer Risk in Sisters of Breast Cancer Patients. Cancer Causes Control 22(7): 1061-66.

Poster

Kim S, Taylor JA, Sandler DP. Prostaglandin E_2 and Postmenopausal Breast Cancer: Design and Characteristics. Department of Defense Era of Hope Meeting. Orlando, Fl, August, 2011

Presentation

Presented a seminar titled "Obesity and Inflammation: from Colorectal Cancer to Breast Cancer" at Georgia Health Science University, Augusta GA (April 26, 2011)

Conclusions

In the past year, the DOD Postdoctoral Fellowship provided the PI with invaluable experience in conducting molecular epidemiologic studies. She designed the experiments and monitored the laboratory performance, and has closely worked with laboratory scientists to improve the quality of the assay, which is a key first step to

address the study questions. The PI has also accumulated knowledge on breast cancer biology through various venues and has incorporated new ideas into the analyses of epidemiologic data, aiming to give new perspectives to old questions. These efforts should provide good preparation for a new position at an academic institution. The laboratory analysis of estrogens is scheduled to complete by the end of April, 2012. In the next five months, the PI will conduct a full analysis to investigate the interrelationships between PGE-M, estrogens and postmenopausal breast cancer risk, in accordance with the approved Statement of Work for this grant.

Tables

Table 1. Within- and between-batch variability of PGE-M measurements, indicated as %coefficient of variation (%CV)

	Creatinine (mg/mL)	PGE-M (ng/mL)	Creatinine-adjusted PGE-M (ng/mg Cr.)
Within-batch %CV[1]	4.0	6.4	8
Batch 1*	1.6	2.6	3.7
Batch 2*	5.6	3.8	8.8
Batch 3*	1.9	6	6.6
Batch 4*	2.1	4.4	5.2
Batch 5*	8.7	5.5	10.8
Batch 9	3	6	9
Batch 14	3	11	14
Batch 19	5	8	10
Batch 22	4.7	5.5	6.7
Batch 25	2	6	5
Batch 28	5	9	7
Batch 33	4.4	8.7	9.2
Batch 38	5.4	6.2	7.5
Between-batch %CV[2]	7.5	13.9	19.1

[1] Based on 50 pairs of quality control samples* and 80 pairs of study samples run in duplicate within the same batch (260 samples)
[2] Based on 12 pairs of replicates analyzed in different batches (24 samples)

Tabe 2. Variance components for PGE-M measurements estimated from linear mixed model[1]

	Between-subject σ^2 (% total variance)	Residual σ^2 (% total variance)	Adjustment factor
PGE-M (ng/mL)	6.975 (60.1)	4.624 (39.9)	None
	7.709 (63.8)	4.382 (36.2)	Batch effect
Creatinine-adjusted	3.335 (42.4)	4.535 (57.6)	None
PGE-M (ng/mg Cr.)	2.916 (41.3)	4.136 (58.7)	Batch effect

[1]Estimated from 100 quality control samples from 12 individuals who provided urine samples at multiple time points over 9 months

Table 3. Factors affecting PGE-M levels (ng/mg Cr.) in 604 study samples, Sister Study 2003-2007[1]

	No.	Mean (95% CI)		P-value	Adjusted mean (95% CI)		P-value
Age							
50-54y	91	5.41	(4.75-6.17)	Ref	5.51	(4.83-6.28)	Ref
55-59y	151	5.52	(4.99-6.11)	0.743	5.51	(4.98-6.1)	0.998
60-64y	191	5.43	(4.96-5.94)	0.903	5.38	(4.92-5.89)	0.78
65-69y	101	5.12	(4.52-5.79)	0.601	5.13	(4.53-5.81)	0.442
70-75y	70	5.53	(4.76-6.41)	0.792	5.53	(4.76-6.42)	0.974
P for linear trend				0.797			0.659
Body mass index							
<25	107	4.84	(4.43-5.28)	Ref	4.98	(4.56-5.43)	Ref
25-29.9	89	5.61	(5.11-6.16)	0.023	5.55	(5.06-6.09)	0.093
30+	100	5.82	(5.34-6.34)	0.003	5.73	(5.25-6.24)	0.028
P for linear trend				0.003			0.028
Smoking history							
Never	315	5.03	(4.69-5.4)	Ref	5.10	(4.75-5.47)	Ref
Past	243	5.52	(5.1-5.98)	0.085	5.46	(5.04-5.92)	0.202
Current	46	7.87	(6.55-9.46)	<0.001	7.68	(6.38-9.25)	<0.001
P for linear trend				<0.001			0.001
Diabetes							
Yes	540	5.28	(5.01-5.58)	Ref	5.30	(5.02-5.59)	Ref
No	64	6.52	(5.57-7.63)	0.014	6.40	(5.47-7.49)	0.026
Cardiovascular diseases							
Yes	558	5.30	(5.02-5.59)	Ref	5.33	(5.06-5.62)	Ref
No	49	6.74	(5.63-8.07)	0.012	6.33	(5.27-7.6)	0.078
Rheumatoid arthritis							
Yes	569	5.47	(5.19-5.77)	Ref	5.47	(5.2-5.77)	Ref
No	35	4.44	(3.59-5.51)	0.064	4.44	(3.59-5.48)	0.058
Diagnosis of breast cancer							
Yes	297				5.26	(4.89-5.66)	Ref
No	310				5.55	(5.17-5.96)	0.306

[1]Coefficients and means were estimated from a linear regression model
[2]Adjusted for all the variables in the table

Table 4a.Lower limit of quantitation (LLOQ, pg/mL) and within-batch %CV[1] for individual estrogen metabolites (EMs) and EMs by pathway

	E1	E2	4-MeOE1	4-MeOE2	4-OHE1
LLOQ	10	10	25	25	25
%CV	2.7	5.6	13.6	15.8	8.1
	2-MeOE1	2-MeOE2	3-MeOE1	2-OHE1	2-OHE2
LLOQ	10	10	25	10	10
%CV	10.1	9.1	11.5	4.7	9.5
	E3	16-ketoE2	16a-OHE1	16-epiE3	17-epiE3
LLOQ	10	10	10	10	25
%CV	9.6	5.3	4.0	4.6	20.4

[1]Based on 3 pairs of 4 replicate samples and 3 pairs of duplicate samples (21 samples)

Table 4b.Within-batch %CV[1] for EMs by pathway

Parent EMs (E1,E2)	2.7
C4-methylated only (4-MeOE1, 4-MeOE2)	15.2
C4-hydroxylated (4-OHE1, 4-MeOE1, 4-MeOE2)	7.1
C2-hydroxylated only (2-OHE1, 2-OHE2)	5.3
C2-methylated only (2-MeOE1, 2-MeOE2)	9.7
C2-hydroxylated (2-OHE1, 2-OHE2, 3-MeOE1, 2-MeOE1, 2-MeOE2)	5.9
C16-hydroxylated	8.4
Total EMs	5.8

[1]Based on 3 pairs of 4 replicate samples and 3 pairs of duplicate samples (21 samples)